PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: A. H. Warfield
PERIOD COVERED: January, 1989

I. TSNA PRECURSORS

A. **Objective**: To determine the precursors of MS TSNA.

B. **Results**: Microencapsulated nicotine bitartrate (NBT) has been received from R.T. Dodge Co. Two different versions were prepared which will release nicotine at different temperatures in order to serve as models of unextracted nicotine, suspected as a precursor of MS NNK. The materials are being characterized. X-ray microscopy shows very little difference between the microencapsulated and untreated NBT crystals except for filaments on the former.

Method development needed to measure ^{14}C-TSNA formation in MS smoke from cigarettes containing ^{14}C-nicotine was continued. Organic and aqueous phases from a buffer extract of MS TPM spiked with NNK, NNN and nicotine which were labeled with ^{14}C were analyzed by TLC and/or HPLC with radiochemical detection. Approximately 10% of the labeled nicotine was found in the methylene chloride (MeCl) phase, the remainder being in the aqueous phase. ^{14}C-NNK and ^{14}C-NNN were just detectable in the MeCl phase after concentration. Application of larger samples to the TLC plate resulted in streaking or diffusion.

Oriental (Or) CEL was sprayed onto DBC Bu filler which had been extracted with 5% EtOH/Hexane in an attempt to determine whether pyrosynthesis could be inhibited in a filler which contains minor alkaloids and nitrate but very little nicotine or endogenous TSNA. However, the resulting filler was sticky and clogged the maker when an attempt was made to prepare cigarettes. OrCEL was also sprayed onto an RL made previously from 1/2 the normal level of BuCEL on Bu base web (BW), yielding 1/2 OrCEL on 1/2 BuCEL on BuBW. A significant reduction in MS TSNA was achieved, but the reduction was not as great as when the CELs were premixed before spraying onto the BuBW, suggesting that chemical or physical interactions result when the CELs are mixed. Experimental RLs have also been prepared to test the role of major salt cations of Or tobacco in the reduction of TSNA observed in the mixed RL study. Data on these RLs will be available shortly.

D. **Plans**: The microencapsulated nicotine will be incorporated into filler and MS smoke data obtained in order to determine whether TSNA are formed. Cigarettes prepared from filler containing ^{14}C-nicotine will be smoked and the MS-TPM analyzed for ^{14}C-NNN and ^{14}C-NNK using methods currently under development. Additional studies with OrCEL on extracted fillers are in progress.

E. **References**:

Haut, S. A. Notebook 8768, p. 15.
Hassam, S. B. Notebook 8712, p. 109.
Hellams, R. D. Notebook 8613, p. 125.

II. ORIENTAL TOBACCO STUDIES

A. **Objective**: To determine if the inhibition of TSNA formation/pyrosynthesis is due to agronomic effects.

B. **Results**: Filler TSNA data for the McNair 373 (bright control) and the three Samsun (oriental) test samples that were grown in Whiteville, N.C., were reexamined, omitting extremely high and extremely low replicates. However, the data still resulted in the conclusion that Samsun tobacco grown under bright conditions and sun-cured produces more endogenous TSNA when not topped than when topped, and much more TSNA when flue-cured than when sun-cured.

A pilot study was initiated to determine whether there is a difference between various types of oriental tobacco in TSNA content and/or MS TSNA delivery. Three types were readily available from another study, and some analytical data were available on these samples: TIU (Turkish Izmir), TSK (Turkish Samsun) and GMU (Greek Macedonium). Filler TSNA data were obtained on these fillers, and alkaloid as well as MS TSNA data are being obtained.

C. **Plans**: Cigarettes have been prepared from fillers obtained in both of the above studies and MS TSNA data are now being obtained. Other data will be obtained from ARD. Interpretation of the results of the U.S. grown oriental study as well as the pilot study of oriental types will be attempted when all the data are complete.

D. **References**:

Keene, C. K. Notebook No. 8754, p. 32.
Haut, S. A. Notebook No. 8768, p. 15.

III. CROSSED SOLUBLES/BASE WEB STUDY (CHEMISTRY)

A. **Objective**: To investigate the smoke chemistry of model cigarettes made from all possible combinations of solubles from bright, burley and oriental tobaccos on base webs from the three tobaccos.

B. **Results**: OrCEL + BuCEL were mixed in equal proportions and sprayed onto BuBW to reproduce the RL previously shown to yield reduced TSNA levels relative to a BuCEL on BuBW control. Appropriate controls were also prepared. The fillers were made into handmade cigarettes and smoked in quadruplicate to collect IT CSCs for submission to Project 6906 for S/M testing. Additional work was done on the electrodialysis equipment to prepare it for service in order to remove specific ions from CEL solutions.

C. **Plans**: The membrane stack will be installed and the electrodialysis equipment run using a standard salt solution. Plans are being formulated to examine the effects of cations, nitrogenous compounds, sugars, and ammonium salts. Further plans will be considered to design synthetic or reconstituted CEL, remove CEL protein, and use glycosidases and peptidases on CEL and BW. Mixed CEL studies are also being planned.

D. **References**:

Hassam, S. B. Notebook 8712, p. 109.
Hellams, R. D. Notebook 8613, p. 125.
McGee, N. H. Notebook 8743, pp. 7-8.

IV. SUPPORT FUNCTION: CONDENSATE PREPARATION

A. **Objective**: To fabricate cigarettes, perform smokings, and prepare condensate as needed for biological and chemical analysis.

B. **Results**: 2R1 IT CSC condensate was collected and processed for testing in the EGF assay. Machine-made cigarettes containing two different levels of encapsulated yeast, along with a control, were smoked in order to obtain IT CSC to be tested in the S/M assay. Hand-made cigarettes were also prepared from three different fillers submitted by M. Parrish.

C. **References**:

Hellams, R. D. Notebook 8613, pp. 124-125.
McGee, N. Notebook 8743, pp. 6,9.

V. EFFECT OF CASINGS ON TSNA DELIVERY:

A. **Objective**: To determine the effect on MS TSNA of casings or flavors normally included in commercial products but not found in reference cigarettes.

B. **Results**: The following samples were smoked for MS TSNA: 1. full flavored blend with PG/G; 2. full flavored blend PGG/G free with Isosweet; 3. RLTC with PG/G; 4. RLTC PG/G free with Isosweet; 5. RL 150-B with PG/G; and 6. RL 150-B PG/G free with Isosweet. The objective here was to determine whether the sugars and/or cooked flavor used in some of these casings have any effect on TSNA delivery. The data obtained showed no effect of the sugars on NNN or NAT deliveries, but the presence of the added sugars correlated with a marginally significant increase in MS NNK. The reason for the increase was not explained by an examination of analytical data obtained on the fillers. There was no difference in MS TSNA delivery attributable to the 150-B.

C. **References**:

Tickle, M. H. Notebook 8716, pp. 151-152.